

***Gliocladiopsis wuhanensis* sp. nov. from China**

NI-PING ZHAI, ZI-QUAN SUN, YA-LONG ZHANG, RUI ZANG,
CHAO XU, YUE-HUA GENG*, MENG ZHANG*

College of Plant Protection, Henan Agricultural University,
95 Wenhua Street, Zhengzhou, Henan 450000, China

* CORRESPONDENCE TO: gengyuehua@163.com, zm2006@126.com

ABSTRACT—A new species, *Gliocladiopsis wuhanensis*, was isolated from soil collected in Hubei Province, China. A combination of ITS, HIS3, and TUB2 DNA sequences were analyzed to evaluate its phylogenetic status. Phylogenetically *G. wuhanensis* is closely related to *G. guangdongensis*. Morphologically, *G. wuhanensis* can be distinguished from related species by its tertiary conidiophore branching.

KEY WORDS—asexual fungi, hyphomycetes, multiple gene, subtropics, taxonomy

Introduction

Gliocladiopsis S.B. Saksena was established with the type *G. sagariensis* S.B. Saksena, characterized by densely penicillate and branched conidiophores (Saksena 1954, Lombard & al. 2015). Crous & Wingfield (1993) synonymized the type species under *G. tenuis* (Bugnic.) Crous & M.J. Wingf. based on morphological studies; however, accurate identification of the *Gliocladiopsis* species based on morphology alone is difficult (Liu & Cai 2013). Through analyzing the ITS, TUB2, His3, 28S LSU, and TEF1- α DNA loci, Lombard & Crous (2012) found *G. sagariensis* and *G. tenuis* phylogenetically distinct and described five new species. Although its teleomorph was described as *Glionectria* Crous & C.L. Schoch (Schoch & al. 2000), the “anamorphic” name *Gliocladiopsis* has nomenclatural priority and is now the accepted generic name under “One Fungus: One Name”. *Gliocladiopsis* currently comprises 14 species (Index Fungorum 2018) mostly soilborne (Lombard & al. 2015) except for

G. guangdongensis F. Liu & L. Cai (Liu & Cai 2013) and *G. aquaticus* Y.Z. Lu & al. (Hyde & al. 2018) isolated from decaying wood in freshwater.

Materials & methods

Isolation

Soil samples from different regions were investigated using a dilution plate method: sterilized water agar medium cooled to 45 °C was mixed with 100 µL ampicillin sodium (100 ng/µL), and poured into a sterile plate with sterile wheat-straw; for each soil sample, three plates were evenly coated with a 200 µL soil suspension diluted 100 times, incubated ca. 3 wks at 25 °C in a biochemical incubator, and observed under stereomicroscope. Single spores were selected and transferred to PDA and synthetic nutrient-poor agar (SNA) (Nirenburg 1981) to obtain pure cultures. The fungi were examined morphologically on slides prepared with lactic acid glycerin. Measurements and descriptions of microscopic structures were made using a Nikon Eclipse 80i light microscope. One isolate was identified as representing *Gliocladiopsis*, from which more than 30 conidiogenous cells and 30 conidia were randomly selected for measurement. A dried culture specimen and living cultures were deposited in the Herbarium of Henan Agricultural University, Zhengzhou, China (HEAC).

DNA extraction, PCR amplification & sequencing

Total genomic DNA was extracted from fresh mycelia using the CTAB method (Doyle & Doyle 1990). PCR amplification primers used were: ITS region—universal primers ITS1 and ITS4 (White & al. 1990); partial β -tubulin gene—T1 (O'Donnell & Cigelnik 1997) and CYLTUB1R (Crous & al. 2004); partial Histone H3 gene—CYLH3F and CYLH3R (Crous & al. 2004). The DNA was amplified using 20 µL volumes containing 1 µL 50 ng/µL genomic DNA, 1 µL each of 10 µM primers, 10 µL Premix Ex Taq (Version 2.0, TaKaRa, containing 0.625 U DNA polymerase, 200 mM dNTP, and 1.5 mM Mg²⁺), and 7 µL ddH₂O. The amplification protocol in an Eppendorf Mastercycler gradient thermal cycler followed an initial denaturation step of 5 min at 94 °C with 35 cycles of 45 s at 94 °C, 30 s at 58 °C, and 1 min at 72 °C and a final elongation step of 10 min at 72 °C concluding with incubation at 4 °C. PCR products were examined on 1% agarose gel and sequenced on an ABI 3730XL automated DNA Analyzer by Sangon Biotech (Shanghai) Co, Ltd.

Sequence alignment & phylogenetic analyses

DNA sequences generated by each primer set were emended using SeqMan v. 7.1.0 in the DNASTAR Lasergene core suite software. Sequences were aligned and edited manually using MEGA v6.0 (Tamura & al. 2013) and then blasted in GenBank. Gene sequences of 39 strains were downloaded from NCBI, including the outgroup *Calonectria brachiatica* (CBS 123700) and *C. brassicae* (CBS 111869). The Bayesian Inference (BI) phylogenetic tree generated with MrBayes v.3.1.2 with the

Markov Chain Monte Carlo (MCMC) consisted of 4 runs (Ronquist & Huelsenbeck 2003), with each run analyzing 4 chains analyzed with 10 million generations (trees sampled every 1000 generations, a sample frequency of 100, stopping when average standard deviation of split frequencies reached below <0.01, and a burn-in of 25% (Huelsenbeck & Ronquist 2001; Ronquist & Huelsenbeck 2003). Trees were viewed in FigTree v. 4.0, then modified using Adobe Illustrator CS6 and saved in Adobe Photoshop in JPG format. Newly generated sequences were deposited in GenBank.

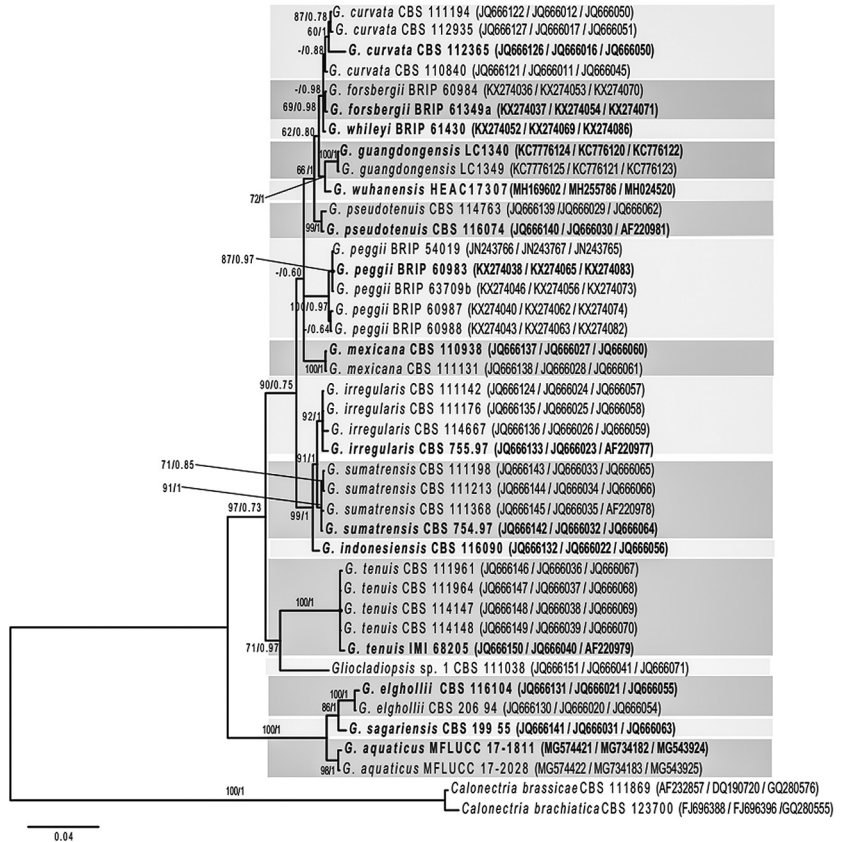


Fig. 1. Maximum-likelihood (ML) *Gliocladiopsis* tree obtained from the combined DNA sequence data from three loci (internal transcribed spacer region, histone H3, and β -tubulin). *Calonectria brachiatica* (CBS 123700) and *C. brassicae* (CBS 111869) were included as the outgroup. Bootstrap support values >50 %, and Bayesian posterior probability values >0.95, are shown at the nodes (BS/PP). Branches supported by BS = 100 % and PP = 1 are depicted as black thickened lines. Ex-type strains are in bold.

Phylogenetic results

Amplicons of approximately 400–600 base pairs were determined for TUB2 (489 bp), HIS3 (477 bp), and ITS (461 bp). The phylogenetic analysis comprised 15 ingroup and two outgroup [*Calonectria brachiatica* (CBS 123700), *C. brassicae* (CBS 111869)] taxa. The combined data set comprised 1427 characters, including 35 gaps and 775 constant characters. Analysis of the 305 parsimony-informative characters yielded one tree (FIG. 1). GTR+I+G was the model selected for Bayesian analysis and Maximum Likelihood (ML). The tree (FIG. 1) divides *Gliocladiopsis* isolates into two main clades, the first (PP = 1) containing the extype *G. sagariensis* strain (CBS 199.55), two *G. elghollii* strains (CBS 206.94, CBS 116104), and two *G. aquaticus* strains (MFLUCC 17-1811, MFLUCC 17-2028), and the second (PP = 0.73) containing the other 12 *Gliocladiopsis* species: (*G. curvata*, *G. forsborgii*, *G. guangdongensis*, *G. indonesiensis*, *G. irregularis*, *G. mexicana*, *G. peggii*, *G. pseudotenuis*, *G. sumatrensis*, *G. tenuis*, *G. whileyi*, and *G. wuhanensis*.) on the main branch.

Taxonomy

Gliocladiopsis wuhanensis Meng Zhang, N.P. Zhai & Y.H. Geng, sp. nov. FIG. 2
MB 825569

Differs from *Gliocladiopsis guangdongensis*, *G. curvata*, *G. forsborgii*, and *G. whileyi* by its tertiary conidiophore branching and its longer conidia.

TYPE: China, Hubei Province, Wuhan, from potato farm soil, 15 Jul. 2017, Niping Zhai (Holotype, HEAC17056 [dried culture]; ex-type living culture, HEAC17307; GenBank MH024520, MH169602, MH255786).

ETYMOLOGY: *wuhanensis*, referring to the city where the type was collected.

COLONIES on the SNA effuse, velvet or powdery, whitish to yellow, reverse green, reaching 6 cm diam. in two wks at 25 °C; mycelium mostly superficial or immersed, hyphae smooth, septate, branched, hyaline, 1.0–5 µm. **CONIDIOPHORES** penicillate, without stipe extensions and terminal vesicles, pale brown, smooth, primary branches aseptate or 1-septate, 13.0–38.5 × 2.0–4.5 µm; secondary branches aseptate, 9.5–30.5 × 2.0–4.5 µm; tertiary branches aseptate, 2.5–13.0 × 1.0–2.5 µm. **CONIDIOGENOUS CELLS** polyblastic, hyaline to light brown, 13.0–37.5 × 2.0–3.5 µm. **CONIDIA** single, smooth, hyaline, cylindrical, straight or slightly curved, 0–1-septate, 13.5–18.5 × 2.0–2.5 µm. **TELEOMORPH** unknown.

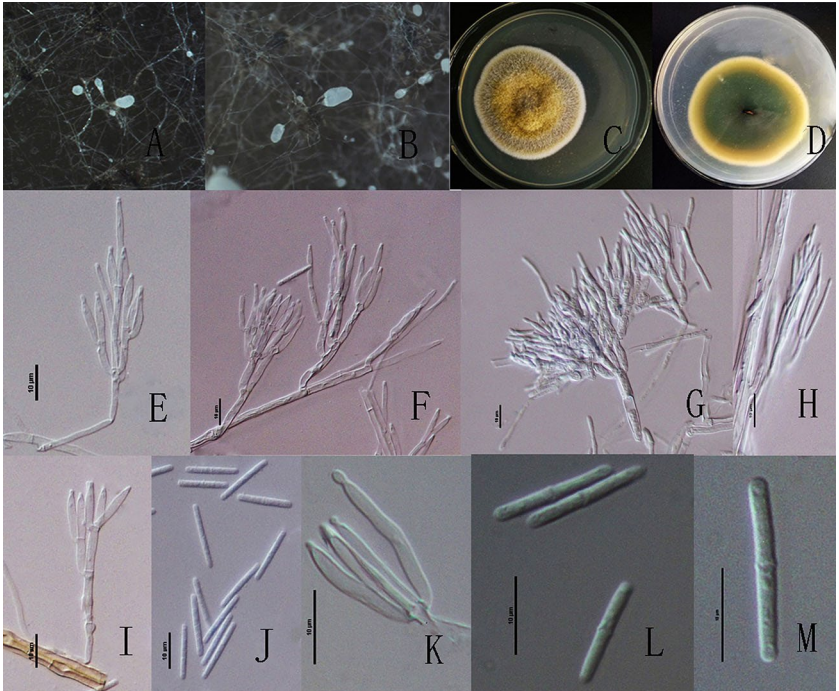


FIG. 2. *Gliocladiopsis wuhanensis* (ex-type culture). A, B. Sporulation phenotype on PDA after 10 d; C, D. Colonies on SNA after 10 d (C = top view, D = reverse view); E–I, K. Penicillate conidiophores; J, L, M. Conidia. Scale bars = 10 µm.

Discussion

Gliocladiopsis wuhanensis is morphologically similar to *G. guangdongensis*, *G. curvata* L. Lombard & Crous, *G. forbergii* L.E. Parkinson & al., and *G. whileyi* L.E. Parkinson & al. However, *G. guangdongensis* differs by its binary conidiophore branching and its shorter conidia (13.5–16 µm; Liu & Cai 2013); *G. curvata* differs by its quaternary conidiophore branching and its shorter, wider conidia (17–21 × 3–5 µm; Lombard & Crous 2012), and *G. forbergii* and *G. whileyi* differ by their quaternary conidiophore branching and their shorter conidia (15.5–19 µm and 17–21 µm; Parkinson & al. 2017).

Phylogenetically, *Gliocladiopsis wuhanensis* is closely related to *G. guangdongensis*. However, for the *HIS3* gene, *G. wuhanensis* is separated by 12 bp from *G. guangdongensis*, greater than that from *G. forbergii* (10 bp) and *G. whileyi* (9 bp), suggesting that they are phylogenetically more distant.

The known *Gliocladiopsis* species were isolated from different habitats (roots of diseased plants, plant litter, water, or soil) (Lombard & Crous 2012). *Gliocladiopsis* species occur quite often in soil associated with plant roots. Dann & al. (2012) conducted tests on plant roots, but the pathogenicity was not clear, and possibly they were mainly saprobic.

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